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Lysis Protein S of Phage Lambda Functions in *Saccharomyces cerevisiae*

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The lambda *S* lysis gene was cloned into a *Saccharomyces cerevisiae* expression vector under *GAL1* control. Induction with galactose in *S. cerevisiae* terminated cell growth and prevented colony formation. Several membrane proteins immunoreactive with anti-S antibody accumulated in the membranes, indicating that sodium dodecyl sulfate-resistant oligomers of S are formed, similar to those observed in the membranes of *Escherichia coli* cells killed by expression of the *S* gene. These observations suggest that the S gene product functions as a cytotoxic protein in the yeast cytoplasmic membrane as it does in the bacterial membrane.

The final event in the life cycle of most bacteriophage is host cell lysis. Lambda lysis requires the function of two genes, *S* and *R*. The *R* gene encodes a transglycosylase specific for the peptidoglycan of *Escherichia coli* and is expressed in at least 100-fold excess over the amount of enzyme required for generalized destruction of the cell wall (1). However, in the absence of *S* function, the *R* transglycosylase accumulates intracellularly without toxicity to the host and lysis does not occur (4, 10, 11). *S* is a 107-codon reading frame that begins with the sequence Met-1–Lys-2–Met-3... and gives rise to two nearly identical polypeptides: S107, resulting from translational initiations at codon Met-1, and S105, resulting from initiations at codon Met-3 (3, 9). These two proteins have opposing functions: S105 is a lethal product that is thought to oligomerize and form a pore in the inner membrane of sufficient size to allow nonspecific escape of the transglycosylase activity to the periplasm, resulting in degradation of the peptidoglycan. In contrast, S107, as a result of the positive charge on the Lys-2 residue, is a

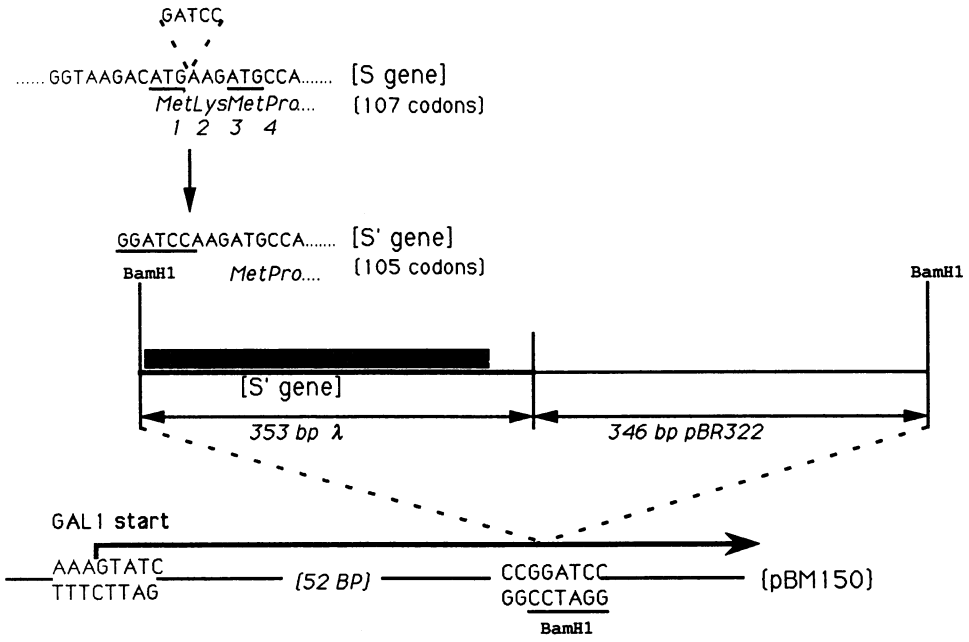

FIG. 1. Construction of pCB3 for *S* expression in *S. cerevisiae*. Site-directed mutagenesis (7) using the primer GGTAAGACATGGATCC AAGATGCCAG was performed on the wild-type *S* subcloned from plasmid pS14 (essentially pBR322 carrying lambda DNA from the *Eco*RI site at lambda map position 44982 to map position 45526, which includes the *S* gene and the first 33 bp of the *R* gene). This resulted in an insertion of a *Bam*HI site between codons 1 and 2 of the *S* gene. The mutant *S* gene, or *S'*, was then subcloned as a *Bam*HI fragment into the yeast *GAL* expression vector pBM150 (6) in an orientation such that the *GAL1* promoter would drive transcription of *S'*.

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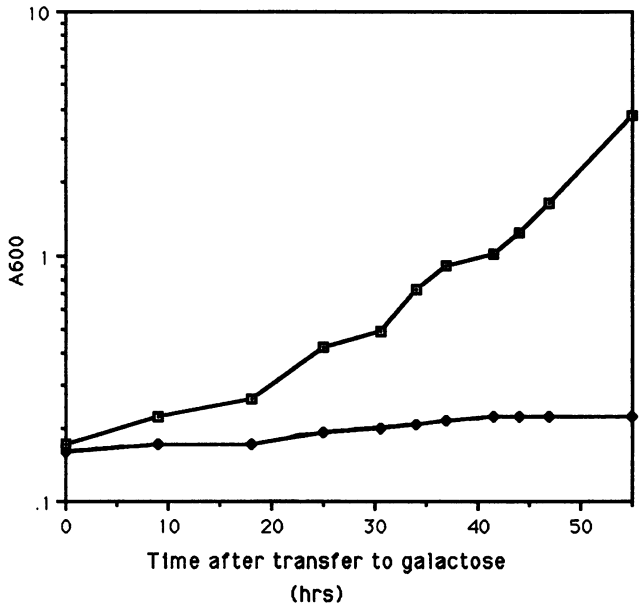

FIG. 2. Toxicity of *S* gene expression in *S. cerevisiae*. Yeast cultures carrying either the *S'* plasmid pCB3 or the vector pBM150 and growing exponentially in glucose-minimal medium were collected and resuspended in galactose-minimal medium at zero time. The galactose cultures were aerated at 30°C, and the A_{600} was monitored. A_{600} values were kept below 0.5 by 10-fold dilutions into prewarmed medium, so the A_{600} levels for the pBM150 cultures reflect correction for two serial dilutions. Under these conditions, at A_{600} = 1.0, there are 2×10^7 cells per ml, or 2 mg (wet weight)/ml.

nonlethal inhibitor of S105 function (2). The partition of *S* expression between killer and inhibitor products determines the kinetics of lysis (3, 8). Nothing is known about the *S* pore. No host mutations conferring resistance to *S* lethal function have been isolated. S protein can be detected in sodium dodecyl sulfate (SDS)-resistant oligomers up to pentamers on polyacrylamide gels (15). These data suggest that the S protein does not require a host target in the inner membrane for nucleation of the pore and thus might be considered a generalized internal cytolysin capable of acting as a lethal factor in eucaryotic cells.

To test this notion, the *S* gene was cloned into a *Saccharomyces cerevisiae* expression vector so that production of the potentially lethal S protein is under the control of the *GAL1* promoter. Site-directed mutagenesis (7) was used to create a *Bam*HI site between codons 1 and 2 of the *S* gene cloned in pBR322 (Fig. 1). A *Bam*HI fragment extending from this newly created site to the *Bam*HI site in the vector was inserted into a unique *Bam*HI site within the *GAL1* promoter sequence of plasmid pBM150 (a *URA3 CEN4 ARS1* shuttle vector with divergent *GAL1* and *GAL10* promoters; described in reference 6). Plasmids with the *S* gene

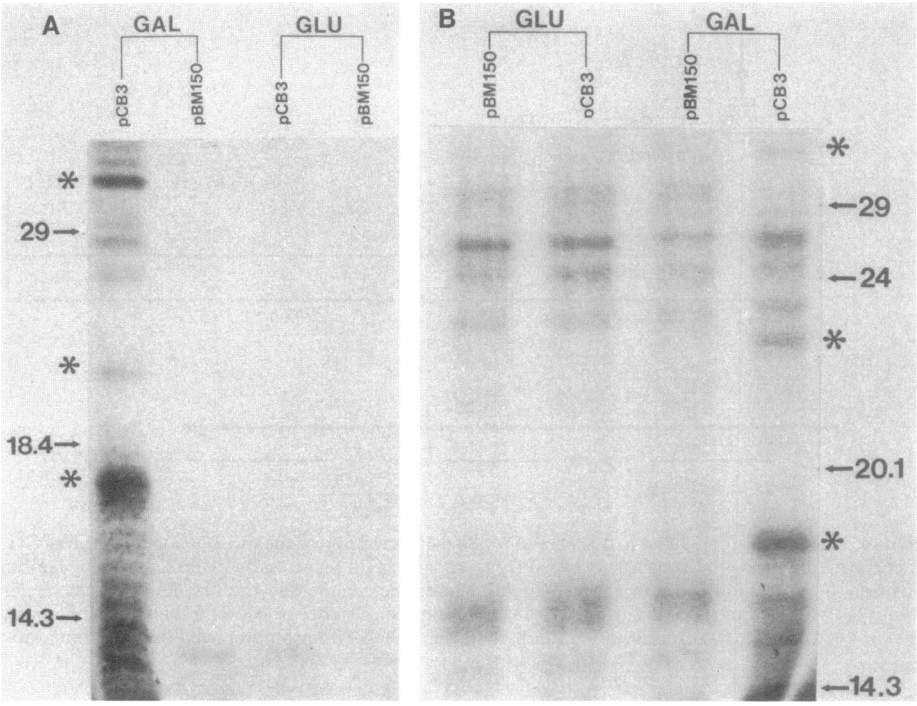

FIG. 3. Accumulation of immunoreactive protein after galactose induction. Cells carrying either the vector (pBM150) or the *S* plasmid (pCB3) were grown in glucose-minimal medium (GLU) and harvested in exponential phase at A_{600} = 0.4 or grown for 35 h after transfer from GLU medium to 5% galactose-minimal medium (GAL; see Fig. 2). Yeast extracts were prepared by concentrating yeast cultures 100-fold and disrupting them with glass beads as described elsewhere (14). Membranes were prepared by centrifugation of the cleared lysate at 12,000 × *g* for 15 min at 4°C; the washed pellet was dissolved at 10 mg of protein per ml by boiling in SDS sample buffer. An SDS-polyacrylamide gel at 10% was used to resolve the membrane proteins; 10 μg of membrane protein was loaded in each lane. The gels were either subjected to immunoblotting (13) with anti-S antiserum (A) or stained with Coomassie blue (B). The anti-S antiserum was a gift of M. Zagotta and D. Wilson and was used as described elsewhere (15).

in either orientation with respect to the *GAL1* promoter were obtained, and one with the correct orientation for *S* expression was selected (pCB3; Fig. 1) and transformed into the *GAL*$^+$ yeast strain SEY6212 (*MATα ura3-52 leu2-3,112 his3-Δ200 trp1-Δ901 suc2-Δ9*) by the alkali cation method (5). *URA3* transformants were purified on minimal-glucose (2%) plates (yeast medium as described in reference 12) and tested for growth on minimal-galactose (5%) plates. Cells carrying pCB3 grew well on glucose-minimal medium but formed only microcolonies on the galactose medium, whereas controls carrying the parental plasmid pBM150 grew as well as wild type on both media. The apparent toxicity of the induction of the *S* gene was confirmed by monitoring growth in liquid culture. The yeast strains carrying pCB3 or pBM150 grew identically in glucose-minimal medium, with a 3-h doubling time (not shown). Yeast cells growing exponentially in minimal-glucose medium were collected, washed with distilled water, and resuspended in minimal-galactose medium. In these conditions, cells cease growth for approximately 24 h after the shift of carbon source while the cells derepress the genes necessary for growth on galactose, including *GAL1*. After this adjustment period, yeast cells containing the parental plasmid resumed exponential growth with a doubling time of 5 to 6 h (Fig. 2). In contrast, yeast cells expressing the *S* gene grew very slowly (doubling time of >24 h) for a maximum of one doubling and then ceased growth altogether (Fig. 2). Dilution into fresh galactose medium did not result in further growth.

Membrane preparations of cells carrying the *S* plasmid or the parental plasmid in repressing or inducing conditions were resolved on SDS-polyacrylamide gels. Several bands visible by Coomassie blue staining were seen in the samples from the galactose-induced cells carrying pCB3 but not in the uninduced or control samples (Fig. 3A). Immunoblot using anti-S antibodies revealed that at least three of these species reacted with the antibody, most prominently at molecular sizes of 17, 22, and 32 kDa (Fig. 3B). SDS-resistant noncovalent oligomers of S have been demonstrated in preparations from *E. coli* membranes (15). Since the S monomer from *E. coli* membranes migrates as species of 8 to 9 kDa, the larger bands observed here may result from a combination of oligomerization and proteolysis. The smallest species exhibited a significant trail of apparent proteolytic degradation products which obscured the lower-molecular-weight region where the S monomer would be expected to migrate.

Thus, expression of *S* in the yeast *S. cerevisiae* resembles *S* expression in the normal host, *E. coli*, in at least two important ways: the production of S protein results in severe toxicity to the cell, effectively terminating growth; and SDS-resistant oligomeric forms of S protein can be detected in the membrane fractions of both kinds of cells. These data are consistent with the notion that the S protein forms oligomeric lesions in the cytoplasmic membrane of yeast cells and suggest that S protein requires no protein targets in the bacterial (or yeast) cell membrane but instead inserts and aggregates spontaneously. In bacteria, approximately 300 molecules of S are required for lysis (9); accordingly, S can be detected in bulk membranes only by immunoblotting. Presumably, the much higher levels of S evident in the yeast membrane fraction reflect the fact that much more S accumulation is required for cell death, because, unlike bacteria, yeast cells do not depend on the plasma membrane for respiration and oxidative phosphorylation. In any case, the fact that Coomassie blue-detectable quantities of S can be seen suggests that it may be possible to obtain purified S protein from yeast membranes in useful quantities, so that current models for S-mediated pore formation can be studied in vitro.

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